REMARKS

Amendments to the Claims

Applicant has canceled Claims 1-22, 24, 29-32 and 34-58 to make the Claims consistent with the Reply to Restriction Requirement, filed January 19, 2001. Applicants have amended Claims 23, 26-28 and 59-62 to correct obvious typographical errors and to recite, "in one or more samples". Support for the amendment can be found throughout the specification, for example, on page 17, lines 22-24, the Specification recites, "Generally, a sample (*e.g.*, any DNA-containing biological sample such as a tissue biopsy, whole blood, isolated cells) is acquired and DNA is isolated from the cells contained in the sample." No new matter has been added. Entry of the amendments is respectfully requested.

Rejection of Claims 61 and 63-65 under 35 U.S.C. §112, first paragraph

The Examiner has rejected Claims 61 and 63-65 under 35 U.S.C. §112, first paragraph, because the Claimed invention is not described in the Specification in a way as to enable one of skill in the art to make and/or use the claimed invention.

Applicants have canceled Claims 61 and 63-65, thereby obviating the rejection.

Withdrawal of the rejection is respectfully requested.

Rejection of Claims 23, 25-28, 33 and 59-65 under 35 U.S.C. §112, second paragraph

The Examiner has rejected Claims 23, 25-28, 33 and 59-65 under 35 U.S.C. §112, second paragraph as being indefinite for failing to point out and distinctly claim that which Applicant regards as his invention.

The Examiner rejects Claims 23, 25-28, 33 and 59-65 for omitting an essential step in determining whether "inherited" refers to Mendelian inheritance, non-Mendelian inheritance or both. However, Applicants state in the Specification: "Inherited point mutations are those that are present in the genome of an individual at conception." (page 16, lines 1-2) Therefore, the term is clear as defined; such mutations are in the genome and present at conception.

The Examiner rejects Claims 23 and 25 for being indefinite because it is unclear whether the method identifies one gene that carries one or more harmful alleles or multiple genes that

carry one or more harmful alleles. Applicants assert that the method can identify both one or multiple genes, hence the use of the "one or more" descriptor. Additionally, the method can identify one or more alleles in each of the one or more genes. Therefore, the claim is clear in that it comprises the identification of one or more alleles in one or more genes. Applicants further note that step (b) is directed to determining the sum frequencies of point mutations for each of the one or more genes. It is not a sum frequency of all point mutations detected. Step (a) identifies frequencies of each point mutation, and step (b) sums these frequencies of point mutations occurring in the same gene.

The Examiner rejects Claims 26-28 as being indefinite over the recitation, "the age-specific decrease of said two or more point mutations." The Examiner also rejects Claims 26-28 for containing terms that lack antecedent basis. Applicants have amended Claim 26, thereby obviating the rejection. Applicants note that Claims 27 and 28 depend from Claim 26.

The Examiner rejects Claim 61 as being indefinite because the preamble does not seem to correspond to the final method step, and because it is not clear how the sums of obligatory knock-out point mutations are calculated. Applicants have canceled Claim 61, thereby obviating the rejection.

In light of the amendments and above remarks, reconsideration and withdrawal of the rejections are respectfully requested.

Rejection of Claims 23 and 62 under 35 U.S.C. §102(b)

The Examiner has rejected Claims 23 and 62 under 35 U.S.C. §102(b) as being anticipated by Kervinen *et al.* (*Atherosclerosis*, 105:89-95, 1994; Reference AT2), as evidenced by Margaglione *et al.* (*Stroke*, 29:399-403, 1998).

Applicants have amended Claims 23 and 62 to recite the identification of one or more inherited point mutations in one or more samples, thereby obviating the rejection as the teachings of Kervinen *et al.* only teach the identification of point mutations through a database search and not in a sample. Kervinen *et al.* do not teach the identification of point mutations in the apolipoprotein E (apoE) gene locus. Kervinen *et al.* demonstrate the determination of allele frequencies of known previously identified alleles. They do not teach identification of any previously unidentified alleles; they merely characterize known alleles. It is the advance of

Applicants disclosed invention that all alleles, whether known or unknown, can be screened from a sample. Point mutations are then actually identified in the sample through the practice of the claimed invention. The advantage of the claimed invention over the teachings of Kervinen *et al.* is that it is not dependent on what is already known regarding a particular gene locus. It can, for example, be used to identify point mutations at an un-characterized or unknown gene locus, or it can, for example, be used to identify rare alleles that had not previously been identified. The teachings of Kervinen *et al.* demonstrate the art at the time of filing was dependent upon taking known or identified alleles and further characterizing them by determining allele frequencies in different populations. Such methods could not allow for a complete sampling of all alleles for a given gene locus for the entire population. The fact that Applicant's claimed invention allows for the sampling of all inherited point mutations at about or above a frequence of 5×10^{-5} represents an improvement of methods known at the time by several orders of magnitude.

In addition, the Examiner points out that the teachings of Kervinen *et al.* do not disclose that the apoE polymorphisms are knock-out point mutations, as claimed in Claim 62. The Examiner states that Margaglione *et al.* indicate the ε4 allele has a Cys->Arg substitution at position 112, however, it is still taught that this allele is an isoform of apoE and, therefore, functional. The Specification, on page 25, lines 10-12, states, "Obligatory knock-outs are point mutations which necessarily inactive the gene, such as point mutations which introduce stop codons or frame shifts in exons of protein encoding genes." A Cys-.Arg substitution is clearly not an introduction of a stop codon or represntative of a framshift mutation. There is no independent evidence that the product encoded by the ε4 allele is non-functional. In fact, the description of the E4 product as an "isoform" of apoE in the Kervinen *et al.* reference is indicative of the fact that the gene is functional. If the ε4 allele is a functional gene, then it cannot be a knock-out mutation.

Applicants note that the teachings of Margaglione *et al.* do not include a teaching that the ε4 allele comprises a knock-out mutation.

In light of the amendments and above remarks, reconsideration and withdrawal of the rejection are respectfully requested

Rejection of Claims 25, 33, 59 and 60 under 35 U.S.C. §103(a)

The Examiner has rejected Claims 25, 33, 59 and 60 under 35 U.S.C. §103(a) as being obvious in light of Kervinen *et al.* (*Atherosclerosis*, 105:89-95, 1994; Reference AT2), Khrapko *et al.* (1)(*Nucl. Acids Res.*, 25:685-693, 1997; Reference AU2) and Khrapko *et al.* (2)(*Nucl. Acids Res.*, 22:364-369, 1994), as evidenced by Margaglione *et al.* (*Stroke*, 29:399-403, 1998).

Applicants respectfully traverse this rejection.

In order to establish prima facie obviousness, the Examiner must show, for example, that the reference or combination of references teaches every limitation of the claims, and that, where references are combined, there is a proper rationale for combining references. (MPEP §2143)

Claims 25, 33, 59 and 60 each clearly indicate that the set of point mutations to be identified is the set of all point mutations occurring at a frequency at about or above 5×10^{-5}. It has been reported that 30 variants of apoE have been characterized, 14 of which are associated with disease (de Knijff, P. *et al.*, 1994, *Hum. Mutat.*, 4:178-194, attached herewith as an Exhibit). Kervinen *et al.* teach detecting allele frequencies of only the three most common apoE alleles, ε2, ε3 and ε4. Thus, Kervinen *et al.* cannot possibly have taught set of all point mutations occurring at a frequency at about or above 5×10^{-5}, since they do not even teach the identification of all of the polymorphisms known at the time. Therefore, Kervinen *et al.* do not teach every limitation of the claims. The limitation of assessing the set of all point mutations occurring at a frequency at bout or above 5×10^{-5} is not taught by the Khrapko *et al.* (1), Khrapko *et al.* (2) or Margaglione *et al.* references. Therefore, the combined references do not teach every limitation of Claims 25, 33, 59 and 60.

Additionally, although the Examiner asserts that the motivation to combine references would have been that combining CDCE with high fidelity PCR permitted detection of low frequency mutations. Applicants note that this would be motivation to combine Khrapko *et al.* (1) with Khrapko *et al.* (2) if more sensitive mismatch detection techniques were desired. It does not provide a basis for combining the two Khrapko *et al.* references with the Kervinen *et al.* reference.

Nowhere in the Khrapko *et al.* references is there a suggestion to use CDCE combined with high fidelity PCR to detect the set of all point mutations for one or more genes in young and old populations. Likewise, there is no suggestion in Kervinen *et al.* that more sensitive point

mutation detection techniques would be useful, as the teachings of Kervinen *et al.*, as noted above, were dependent on previously characterized alleles. Kervinen *et al.* teach the determination of frequencies of known alleles and do not identify the alleles themselves through direct laboratory analysis.

In light of the above remarks, reconsideration and withdrawal of the rejection are respectfully requested.

Information Disclosure Statement

Information Disclosure Statements (IDS) were filed on June 12, 2000 and February 5, 2001 and a Supplemental Information Disclosure Statement (SIDS) is being filed concurrently herewith. Entry of the SIDS is respectfully requested.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned.

Respectfully submitted,

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